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Global Atmospheric Change: Potential Health Effects of Acid Aerosol and Oxidant Gas Mixtures

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Inhalation toxicology experiments in whole animals have demonstrated a remarkable lack of toxicity of sulfuric acid in the form of respirable aerosols, especially in rats and nonhuman primates. Thus, much of the current experimental emphasis has shifted to the evaluation of the potential health effects of acid aerosols as components of mixtures. Rats have been concurrently exposed to mixtures of ozone or nitrogen dioxide with respirable-sized aerosols of sulfuric acid, ammonium sulfate, or sodium chloride, or to each pollutant individually. Their responses to such exposures have been evaluated by various quantitative biochemical analysis of lung tissue or wash fluids ("lavage fluid") or by quantitative morphological methods ("morphometry"). Such studies have mainly been performed in the acute time frame due to the inherent limitations of the most sensitive assays available and have generally involved exposures for 1 to 9 days, depending on the assays used. Good correlations were found between the most sensitive biochemical indicators of lung damage (protein content of lung lavage fluid or whole lung tissue and lung collagen synthesis rate) and the exposure concentration of oxidant gas present alone or in mixtures with acidic aerosols showing interactive effects.

Synergistic interaction between ozone and sulfuric acid aerosol was demonstrated to occur at environmentally relevant concentrations of both pollutants by several of the analytical methods used in this study. Such interactions were demonstrated at concentrations of ozone as low as 0.12 ppm and of sulfuric acid aerosol at concentrations as low as 5 to 20 $\mu g/m^3$. The acidity of the aerosol is a necessary (and apparently a sufficient) condition for such a synergistic interaction between an oxidant gas and a respirable aerosol to occur. A hitherto unexpected synergistic interaction between nitrogen dioxide and sodium chloride aerosol was found during these studies; it is hypothesized that this was due to formation of their acidic (anhydride) reaction product, nitrosyl chloride, in the chambers during exposure to the mixture.

Introduction

One of the major global atmospheric changes associated with increased industrialization, and especially with increased combustion of fossil fuels, is the release of acids and their precursors to the atmosphere. Major concerns in this area involve acids arising from SO_x and NO_x emissions, including sulfuric acid and bisulfate aerosols. Thus, we might well ask what is known of the toxicological impacts of acid aerosols. In this manuscript I review and discuss specific data bearing on this question based on experiments performed in animals. I consider effects of acid aerosols alone and in combination with ozone and other atmospheric oxidants.

Effects of Sulfuric Acid Aerosols on Lungs of Experimental Animals

Guinea pigs seem to be particularly susceptible to sulfuric acid aerosols compared with other laboratory animals. Guinea pigs respond by showing increased airway resistance after exposure for 1 hr to 100 to 1000 $\mu g/m^3$ (*1*). Interestingly, no long-term effects of exposure of guinea pigs to 100 $\mu g/m^3$ for 52 weeks were reported by Alarie and co-workers (*2*).

Rats or monkeys are remarkably resistant to observable effects on lung when acutely exposed to respirable aerosols of sulfuric acid (*3–5*), even at concentrations of acid at or above 50 to 100 mg/m^3. On the other hand, guinea pigs and, to a lesser extent, mice, respond to exposure to lower concentrations of sulfuric acid mist (*1,4,5*) and presumably represent susceptible species to this agent. Intermittent exposure of mice to 1.4 mg/m^3 of sulfuric acid mist in conjunction with carbon particles (1.5 mg/m^3; no acid-alone group was exposed) for 20 weeks caused decreased resistance to experimental pulmonary infections (*6*). No effects of sulfuric acid aerosol exposure at 8 mg/m^3 in dogs and at 4 to 14 mg/m^3 in sheep were observed for various cardiopulmonary functions examined (*7*). The relatively high resistance of experimental animals to exposure to sulfuric acid aerosol is assumed to reflect its high absorption by the nasopharynx, thereby reducing the dose reaching the distal lung to very low levels. This is consistent with its high solubility in water. However, sulfuric acid aerosol exposure at relatively low concentrations has been associated with alterations in mucociliary clearance rates for various tracer substances introduced into respiratory airways of experimental animals. Fairchild et al. (*8*) reported that exposure of guinea pigs for 1 hr to concentrations

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of acid between 30 and 3000 $\mu g/m^3$ caused a proximal shift (upwards in the respiratory airways toward nasopharynx and trachea) in the sites of deposition of radioactively labelled streptococcus administered by aerosol. Rabbits exposed for 1 hr to 200 to 1000 $\mu g/m^3$ of sulfuric acid aerosols show altered rates of mucociliary clearance of radioactive tracers (*9,10*), as do donkeys (*11*).

Chronic exposure of monkeys for up to 78 weeks to sulfuric acid aerosol at 1 to 5 mg/m^3 resulted in histological changes to the bronchial mucosa and changes in epithelial cells of bronchi and respiratory bronchioles (*2,3*). These results are consistent with the known sites of deposition for particles of about 0.5 to 5 μm, which are preferentially deposited in the deep lung (*12*).

Effects of Ozone on Lungs of Experimental Animals

Acute exposure to high concentrations of ozone, in excess of about 1 ppm, causes severe pulmonary edema and hemorrhage, which can result in death of experimental animals at concentrations in excess of 1.5 ppm for periods of several hours or days. However, with concentrations at or below approximately 1.0 ppm of ozone, effects of ozone related to edema and cellular inflammation are more subtle and are reflected as increased lung weight and as increased lung content of, or lung enzyme activities of, a large variety of measurable parameters and enzymes associated with cellular inflammation and edema (*13–17*).

The major changes recorded in experimental animals exposed to moderate concentrations of ozone (below 1 ppm for hours or days) include damage to respiratory tract epithelium, especially loss of cilia from ciliated cells and cell necrosis. The two major sites of damage appear to be loss of cilia from epithelial cells of the trachea and large bronchi and epithelial cell necrosis in the centriacinar region of the lung. Particularly susceptible cells seem to include the alveolar type I cell and the Clara cells (*16*). Other changes observed in animals exposed to moderate concentrations of ozone include those typical of an inflammatory cell response, especially in the epithelial layer of small bronchioles in the centriacinar region (*16*). Chronic exposure to ozone is associated with a continuing bronchiolitis and continuing inflammatory response of the centriacinar region (*18*). Another chronic effect of exposure of animals for long periods of time to moderate levels of ozone is pulmonary fibrosis; that is, the accumulation of collagen in the centriacinar region of the lung (*19–21*). These results are consistent with the predicted sites of maximal deposition of ozone in the respiratory tract based on its known relative solubility in water and established models of ozone dosimetry to the lung (*12*).

Several laboratories have reported changes in the protein content of lung lavage fluid in animals exposed to ozone. Hu et al. (*22*) reported increased accumulation of protein in lung lavage fluid from guinea pigs exposed for 72 hr to 0.26, 0.51, or 1 ppm of ozone. No effect was observed at 0.10 ppm. Guth et al. (*23*) extended these studies by examining concentration × time relationships. The increased protein in lavage fluid seems to originate from serum, suggesting that the changes being measured arise from pulmonary edema. Costa et al. (*24*) reported that rats exposed to ozone had a higher alveolar permeability to serum albumin than did normal rats. Frank et al. (*25*) also reported pulmonary edema in rabbits exposed to high quantities of ozone for short periods of time.

Bartlett et al. (*26*) reported decreased lung tissue elasticity consistent with mild pulmonary fibrosis in young rats exposed to 0.2 ppm of ozone for 30 days. Several groups have reported increased collagen synthesis by lungs of experimental animals exposed to ozone (*27–30*). The significance of these observations with regard to the etiology of pulmonary fibrosis is controversial. Filipowitz and McCauley (*30*) have suggested that despite the increase in collagen synthesis rate observed from lungs of rats exposed to ozone, there is no net accumulation of total lung collagen, suggesting increased degradation of collagen accompanying (and offsetting) the increase in synthesis.

Effects of Combinations of Ozone and Sulfuric Acid Aerosol on Lungs of Experimental Animals

Gardner et al. (*31*) reported that mice exposed sequentially to 0.1 ppm of ozone for 3 hr followed by 900 $\mu g/m^3$ of sulfuric acid aerosol for 2 hr showed significantly higher mortality when challenged with an aerosol of *Streptococcus pyogenes* than did mice challenged with either pollutant alone. Osebold et al. (*32*) reported enhanced antigenicity of protein introduced into the respiratory tract in animals exposed to ozone plus sulfuric acid as compared to mice exposed to either agent alone. They interpreted their findings as indicative of increased epithelial permeability, that is, an enhanced effect of ozone, in animals exposed to the mixture of gas and aerosol.

Last and Cross (*33*) reported synergism of ozone effects on rat lungs by sulfuric acid aerosol during simultaneous exposure to concentrations of about 1 mg/m^3 of the acid aerosol and 0.4 ppm of ozone for 3 to 14 days. Parameters evaluated included rate of secretion of mucous glycoproteins by tracheal explants and lung content of water, DNA, RNA, and of various lysosomal enzymes. This same laboratory later showed an enhanced effect of ozone in lungs of rats simultaneously exposed to ozone plus sulfuric acid aerosol as evaluated by elevated lung collagen synthesis rates and morphometric evaluation of lung and inflammatory cell populations at sites of lung lesions (*34,35*). In all of these studies, sulfuric acid aerosol alone had little or no effect on the parameters being quantified; rather, the acid aerosol significantly increased the effect of a given concentration of ozone, as if it were increasing the effective dose of ozone delivered to the centracinar region of the lung. Juhos et al. (*36*) also reported histological changes in lungs of small numbers of rats exposed to 0.9 ppm of ozone plus 2 mg/m^3 of sulfuric acid aerosol, consistent with the observations (*34,35*) described above.

There have also been negative reports of results of exposures to mixtures of ozone and sulfuric acid aerosols. Cavender et al. (*5*) reported no effects in rats or guinea pigs exposed for up to 7 days to 2 ppm of ozone and/or 10 mg/m^3 of sulfuric acid aerosol other than those ascribed to ozone alone. It is, however, noteworthy that control animals lost weight during these exposures, suggestive of illness or other handling problems, and that 2 ppm of ozone for 7 days, which is well in excess of the rat LD_{50} dose, provoked no mortality in this study. Human subjects exposed sequentially to 0.3 ppm of ozone for 2 hr followed by 100 $\mu g/m^3$

sulfuric acid aerosol for 4 hr showed no effects by pulmonary function testing (*37*).

A hitherto unexpected synergism between the oxidant air pollutants ozone or nitrogen dioxide and a respirable-sized aerosol of ammonium sulfate [$(NH_4)_2SO_4$] was observed during controlled exposure of rats to these substances. Response of rat lungs to these pollutants was quantified by measurement of apparent collagen synthesis rates *in vitro* by lung minces from exposed animals. Dose–response curves to either O_3 or NO_2 were altered in the presence of 5 mg/m^3 of $(NH_4)_2SO_4$ aerosol. Morphometric and histologic observations of lungs from rats exposed to high levels of ozone, with and without concurrent exposure to the $(NH_4)_2SO_4$ particles, confirmed such synergistic effects. In a separate set of experiments, rats were exposed to mixtures of ozone and sulfuric acid aerosol (submicron-sized aerosol). Potentiation of ozone effects on lung collagen synthesis rates was also observed in these experiments (*34*).

In subsequent studies, cellular populations were examined at lesions in lungs of rats exposed for 3 or 7 days to 0.64 to 0.96 ppm of O_3 +5 mg/m^3 of ammonium sulfate aerosol (*35*). These studies gave rise to a hypothesis that the synergistic interaction between ozone and acid aerosols may be mediated by changes in the pH of the lung lining fluid caused by local deposition of acid at or near sites of reaction of O_3 or NO_2 with molecules within lung cells or lining fluid.

Subchronic Response of Rat Lungs to Ozone-Sulfuric Acid Aerosol Exposure

An experiment has been performed to examine the potential relevance of ozone-sulfuric acid interaction beyond the acute time frame (1–9 days) studied in our earlier cited experiments. We examined the protein content of lungs from rats exposed for 15 or 30 days to 0.2 ppm of ozone + 1 mg/m^3 of sulfuric acid aerosol, to ozone or acid aerosol alone, or to filtered air. The results of this experiment are shown in Table 1. There was a significant increase in the protein content of the lungs of rats exposed to ozone after 15 or 30 days of exposure. The relative response was similar at both time points for both ozone alone and for ozone plus acid aerosol. The response to ozone plus acid aerosol was significantly greater than that to ozone alone at 15 days; the difference between the animals exposed to ozone plus acid aerosol and ozone alone was not significant at the 30-day time point, although a trend was apparent. We conclude that total lung protein content may be a suitable measurement of lung alteration (damage and/or repair) during the (sub)chronic time frame to pursue some of the remaining questions about ozone–acid aerosol interaction in future studies. Much more work remains to be done, however, before any conclusions can be drawn regarding the occurrence of such an interaction in the (sub)chronic time frame.

Table 1. Exposure of rats for 15 or 30 days to 0.2 ppm of ozone ± 1 mg/m^3 of sulfuric acid aerosol.[a]

Exposure conditions	Lung protein content, mg/lung	
	15 Days	30 Days
Control	151.3 ± 5.0 (100)	173.0 ± 8.7 (100)
Ozone	164.7 ± 1.2 (109)	188.0 ± 11.8 (109)
Acid aerosol	153.6 ± 1.2 (102)	175.6 ± 11.7 (102)
Both	176.2 ± 1.2 (116)	194.7 ± 11.0 (112)

[a]Data are mean values ±SD (n = 6 rats per group) for total lung protein content of rats for the various exposure regimens. Calculations were based on values determined using four replicate 100-μL samples from 4 mL of total homogenate of the right middle plus right lower lobes of rat lung. Each 100-μL sample was precipitated with Cl_3CCOOH and centrifuged, and the pellet was frozen. Protein was then solubilized in 0.5 N NaOH (37°C, 24 hr). NaOH was neutralized with 0.5 mL of 0.5 N HCl. Protein in each sample was determined in duplicate (*49*). The protein contents for each homogenate were obtained by averaging the four sample protein values (eight determinations). Values in parentheses are percent of controls.

Importance of Aerosol Acidity

Studies of the effects of exposure of rats to various aerosols, alone and in combination with high concentrations (0.96 ppm) of ozone, had shown synergistic interaction between ozone and 5 mg/m^3 of ammonium sulfate (pH of stock solution = 5.1), with no interaction observed between ozone and sodium chloride or sodium sulfate aerosols (pH of stock solutions = 7.0) (*38*). These results suggested that the acidity of an aerosol might be the determinant factor as to whether or not it exhibited a synergistic interaction with ozone, and this prompted a series of experiments to more precisely evaluate the role of aerosol acidity in predicting the response of rats to exposure to mixtures of oxidant gases and aerosols.

We have examined the pH and sulfate concentration of aqueous extracts from filters used to trap sulfuric acid aerosols upon sampling of chamber atmospheres during the course of our experiments, including several of the exposure concentration response experiments discussed above. The results of these measurements are shown in Table 2. The apparent pH of filters used to sample filtered air-only exposure chamber samples as measured using these procedures was 4.8. This pH value is similar to aqueous washes of unused filters [mean ± 1 SD was 4.62 ± 0.82 (N = 5)]. As the chamber concentration of sulfuric acid aerosol was increased, the pH of the eluate from the filter extract was decreased. When the mean values for analytically determined sulfate mass concentration were plotted against the mean pH values for samples from atmospheres containing 0.1 to 1.0 mg/m^3 of sulfuric acid aerosol, a linear relationship was apparent (slope = −0.93, r = 0.98). Interestingly, the pH of the samples from chambers containing 40 μg/m^3 of sulfuric acid aerosol or less were slightly less acidic than the value calculated from linear extrapolation of the data from the higher concentrations of acid aerosol (for example, expected and observed values for 40 μg/m^3 aerosol were 3.74 and 4.27, respectively).

These observations suggest two important conclusions. First, only moderately to strongly acidic aerosols result in a synergistic interaction of lung damage with O_3. Based upon the methods for

Table 2. pH measurements of sulfuric acid aerosol samples.[a]

Nominal concentration, mg/m^3	n	Actual concentration, mg/m^3	pH
0 (filtered air)	5	—	4.80 ± 0.14
0.005	12	0.0045 ± 0.0008	4.44 ± 0.08
0.02	12	0.0194 ± 0.0030	4.35 ± 0.08
0.04	31	0.047 ± 0.010	4.27 ± 0.46
0.1	26	0.10 ± 0.03	3.73 ± 0.035
0.5	18	0.60 ± 0.08	3.15 ± 0.07
1.0	26	0.97 ± 0.22	2.93 ± 0.08

[a]Aerosols were generated as described in detail elsewhere (*29,48*).

measurement of aerosol pH used here, only those aerosols possessing a pH of less than approximately 4.4 to 4.5 have been shown to interact synergistically with O_3. Second, other workers have suggested that the pH of chamber atmospheres used for animal exposures might be altered by the presence of ammonia produced by animal metabolism or by breakdown of excreta by bacteria (*39–41*). The correlation coefficient of 0.98 between acid aerosol concentration down to 100 $\mu g/m^3$ of acid aerosol and pH of sampling filter eluates in our experiments suggests that any such neutralization of acid by ammonia in our experiments (down to a sulfuric acid aerosol concentration at or below 0.1 mg/m^3) must have been negligible.

Response to NO_2

Groups of rats were exposed for 7 days to 10, 5, or 2 ppm of NO_2. Rates of lung collagen synthesis as compared with control animals exposed to filtered air were 210, 120, and 99%, respectively. The values at 5 and 10 ppm of NO_2 were significantly increased as compared with the controls. Thus, there was a measurable concentration–response relationship by this assay, with an apparent no-effect level at 2 ppm of NO_2.

Protein content of lung tissue after 7 days of exposure was significantly increased, to 122% of control values, at 10 ppm of NO_2. Values observed at 5 and 2 ppm were, respectively, 98% and 109% of controls; neither value was significantly different from the controls. Thus, an apparent no-effect level, as defined by this assay, was observed at 5 ppm of NO_2.

Response to NO_2 Plus Sulfuric Acid Aerosol

Additional experiments were also performed, based upon our observed results with ozone–acid aerosol mixtures, to ascertain whether a similar synergistic interaction occurred when NO_2 was substituted for ozone. Rats were exposed for 1 or 7 days to 5 ppm of $NO_2 \pm 1$ mg/m^3 of sulfuric acid aerosol. When rats were exposed to $NO_2 \pm$ acid aerosol, protein content of lavage fluid was significantly increased, as compared to values in rats exposed to NO_2 or sulfuric acid alone, to 215% of control values after one day of exposure. Lung collagen synthesis rates, measured after 7 days of exposure, were significantly increased in rats exposed to NO_2 alone (120% of controls) and not affected by acid aerosol alone (98% of controls); response to NO_2 plus acid aerosol was 145% of controls, significantly greater than the response to NO_2 alone. Rats exposed to 2 ppm of NO2 $\pm$ 1 mg/m^3 of sulfuric acid aerosol for 7 days showed the following results as compared to filtered air controls: NO_2 alone, 99%; sulfuric acid alone, 98%; NO_2 plus acid aerosol, 129%, a significant increase. We conclude that the no-observable effect level for response of rat lungs to exposure to NO_2 plus 1 mg/m^3 of sulfuric acid aerosol is below 2 ppm.

Other groups of rats were exposed for 7 days to filtered air, 5 ppm of NO_2, 1 mg/m^3 sulfuric acid aerosol, or NO_2 plus acid aerosol (Table 3). For all parameters evaluated (Table 3), acid aerosol alone was indistinguishable from filtered air controls, while NO_2 alone significantly increased the observed lung inflammatory lesion. The combination of NO_2 plus acid aerosol showed significantly higher values than those observed upon exposure to NO_2 alone.

Table 3. Morphometric analysis of 7-day exposures to 5 ppm of $NO_2 \pm 1$ mg/m^3 of two different aerosols.[a]

Number of rats	Exposure group	V_{lesion}, mm^3	$V_{v\text{-}lesion}$, $\times 10^3$	P_{lesion}, $\times 10^3$
6 (3 + 3)	Air or H_2SO_4 aerosol alone	20.4 ± 8.8	1.8 ± 0.7	100 ± 43
6	5 ppm NO_2	155.9 ± 18.2*	13.0 ± 1.1*	765 ± 89*
6	NO_2 + 1 mg/m^3 H_2SO_4 aerosol	174.8 ± 22.5*	15.2 ± 2.1†	858 ± 110†
6	NO_2 + 1 mg/m^3 NaCl aerosol	179.8 ± 17.4†	15.2 ± 1.0†	882 ± 85†

[a]Data are expressed as mean values ± 1 SD. V, volume; V_v, volume density; P, proportion.

*Significantly greater than air or H_2SO_4 group at $p \leq 0.05$ by Duncan's multiple comparison test.

†Significantly greater than 0.5 ppm of NO_2 or air or H_2SO_4 at $p \leq 0.05$ by Duncan's multiple comparison test.

NO_2–NaCl Interaction

There is no observable interaction as evaluated by responses of lungs of rats exposed to ozone (0.96 ppm) and to respirable aerosols of 5 mg/m^3 of sodium chloride (NaCl) under conditions identical to those that we demonstrated had elicited a synergistic interaction in our experiments with ozone and ammonium sulfate aerosols (*38*). Rats exposed to 5 ppm of NO_2 for 7 days showed a significant increase in lung collagen synthesis rate (120% of control values). Groups of rats exposed to 5 ppm of NO_2 and 1 mg/m^3 of sulfuric acid aerosol had lung collagen synthesis rates of 145% of the control values, i.e., rats exposed to filtered air, values significantly greater than those observed with the rats exposed to NO_2 alone, as reported above (exposure concentration-response experiments). Of interest here, however, is the response of rats exposed for 7 days to 5 ppm of NO_2 + 1 mg/m^3 of NaCl aerosol. These animals showed a lung collagen synthesis rate that was 165% of the values observed with controls exposed to filtered air. In control experiments we found values of 98 and 95% of control for rats exposed to sulfuric acid aerosols and NaCl aerosols alone, respectively, by this assay.

We also quantified the protein content of the lung lavage fluid from rats exposed for 3 days to 5 ppm of NO_2, with and without either 1 mg/m^3 of H_2SO_4 or NaCl aerosol. As compared with filtered air controls, we found values of 175% of controls in the rats exposed to NO_2 alone, 180% of controls in rats exposed to NO_2 plus H_2SO_4 aerosol and 210% of controls in rats exposed to NO_2 plus NaCl aerosol. The increase in values for the NO_2 plus NaCl group was significant as compared to those observed in rats exposed to NO_2 alone (*42*). As shown in Table 3, the group exposed to NO_2 and NaCl aerosol also showed significantly greater morphometric changes than were observed in the rats exposed to NO_2 alone.

We interpret these results as suggesting that a reaction product of NO_2 and NaCl is responsible for the interaction observed in these experiments because no interaction was observed between NaCl and ozone, which do not react chemically. We hypothesize that the interaction between NO_2 and NaCl is due to their reaction to form nitrosyl chloride (NOCl), the mixed anhydride of hydrochloric, nitrous, and nitric acids, which could give rise to strong acids in the centriacinar region of the lung upon hydrolysis (*42–44*). The possibility of acid aerosols (or acidogenic aerosols) arising in the atmosphere from sources other

than SO_2 or direct dissolution of NO_2 to give nitric and nitrous acids opens some very interesting vistas toxicologically.

Conclusions

The most important conclusions of the studies described here are *a*) ozone–acid aerosol interaction occurs in rats at concentrations of each agent that approximate to actually encountered ambient levels; *b*) several sensitive assays may be used to quantify the acute response of the lung to oxidants, alone and in combination with respirable acid aerosols; *c*) there is a reasonably good correlation between the most sensitive biochemical and morphometric indicators of lung response studied; and, *d*) acidity of an aerosol is apparently a necessary and sufficient condition for it to interact synergistically with an oxidant gas to cause increased lung damage.

Table 4 summarizes the results of exposure concentration–response studies. The matrix of ozone concentration, sulfuric acid aerosol concentration, and assays performed gives rise to several conclusions. At the highest ozone concentration examined (0.64 ppm), all of the assays tested show significant increases in lungs of rats exposed to ozone alone. A synergistic interaction between 0.64 ppm of ozone and all three concentrations of sulfuric acid aerosol tested was observed by the criterion of increased lung collagen synthesis rate. Only at the highest concentration of acid aerosol tested, .1 mg/m³, was such a synergistic interaction observed by assay of lung protein content. No significant interaction was observed by assay of protein content of lung lavage fluid at 1 mg/m³ of acid aerosol. We interpret these observations as suggesting that the total protein and the whole lung protein content assays cannot discriminate between the lung damage caused by O_3 and that by O_3 plus acid aerosol exposures at high concentrations of O_3.

Total lavagable protein and total lung protein were significantly elevated above control values after rats were exposed to 0.20 ppm of O_3. Lung collagen synthesis rates were significantly elevated above control values after exposure of rats to 0.20 ppm of O_3 in most of our experiments. A trend toward higher values was observed in the remainder of these experiments. We have previously reported significant increases in lung collagen synthesis rate when rats were exposed to 0.20 ppm of O_3 for 7 days (*45*). A significant synergistc interaction was observed between 0.2 ppm of ozone and all of the acid aerosol concentrations tested by essentially all of the assays performed, consistent with our interpretation that such interaction is most easily evaluated under conditions of minimal lung damage by ozone alone.

Table 4. Summary of results examining ozone sulfuric acid aerosol interaction.

Ozone concentration, ppm	H_2SO_4 aerosol, mg/m³	Assay[a] Collagen synthesis rate	Protein content of lung lavage fluid	Lung protein content
0.64	1.0	+	−	+
	0.5	+	ND	−
	0.2	+	ND	−
	0.0	+	+	+
0.2	1.0	+	+	±
	0.5	+	+	+
	0.1	+[b]	+	+
	0.04	+	−	+[b]
	0.02	ND	−	+
	0.005	ND	−	±
	0.0	±	+	+
0.12	0.5	±	±	−

[a](+) Synergistic interaction observed; (−) interaction not observed; ND, not done; (±), trend observed, but not statistically significant. Values with 0 acid aerosol (i.e., ozone alone) are indicated as (+), (−), or (±) for significant increase, no increase, and trend, respectively, upon exposure to ozone as compared with filtered air controls.

[b]May be either an additive or synergistic interaction.

Rats were exposed to 0.12 ppm of O_3, the current peak hourly National Ambient Air Quality Standard for O_3, with and without 0.5 mg/m³ of sulfuric acid aerosol. Total lavagable protein was the only parameter measured that was significantly elevated for the group exposed to 0.12 ppm of O_3 alone. Collagen synthesis rate was significantly elevated in lungs of rats exposed to 0.12 ppm of O_3 plus 0.5 mg/m³ of sulfuric acid aerosol. Thus, concentrations of O_3 as low as 0.12 ppm may elicit responses from lungs of exposed rats and may be potentiated by concurrent exposures to acid aerosols.

It is not clear whether the small changes in total lavagable protein and in lung collagen synthesis rate observed in this experiment are necessarily predictive of long-term damage. In animals exposed to higher concentrations of O_3 (0.40 ppm), morphological lesions have been shown to persist in monkey lungs during prolonged exposures and for a 3-month period after termination of exposure. Measurements in rat lungs after exposure to 0.64 ppm of O_3 for 1 week showed increases in apparent collagen synthesis rate and in histologically observable collagen consistent with O_3-induced fibrosis. The connection (if any) between the early effects of exposure to 0.12 ppm O_3 shown here and long-term inflammatory and fibrotic changes has not yet been demonstrated. Based upon results observed after exposure of rats to higher levels of O_3, it might be prudent to further examine the possibility that these early changes are indeed predictive of long-term effects of ozone on the lung at frequently encountered ambient concentrations.

We can also conclude from these experiments that assays of enzyme activity in cell-free lavage fluid (*46,47*) are a relatively insensitive method of measurement of ozone-induced lung damage. Acid phosphatase and β-*N*-acetylglucosaminidase enzyme activities failed to show significant changes at concentrations of ozone below 0.40 ppm (24 hr); lactate dehydrogenase activity was an even less sensitive assay, with significant increases found only after exposure of rats to 0.64 ppm of ozone for 24 hr. Preliminary experiments suggested that sialic acid content of lavage fluid was also an insensitive (and highly variable) measurement of ozone-induced lung damage, so this assay was not further examined. Increased movement of labeled tracer from blood to a lavagable compartment of the lung was a more sensitive index of lung damage then were any of the enzyme activity assays. The most sensitive indicator of ozone-induced lung response in lavage fluid was an increase in the total protein content (predominantly serum albumin) of the lung lavage fluid, which was significantly increased above control values after 1 or 2 days of exposure of rats to 0.12 ppm of ozone (*23,48*). Interestingly, the assay of movement of labeled [^{3}H]albumin from blood to lung lavage fluid does not show any synergistic interaction between ozone and acid aerosols under conditions where ozone alone provokes a positive response and where the assay of total lavagable protein content shows synergistic interaction between the mixture of pollutants. It

Table 5. Total lung protein in rats exposed to 0.2 ppm of ozone ± sulfuric acid aerosol for 9 days.[a]

Exposure conditions	Lung protein content, mg/lung	*p*-value versus filtered air control	*p*-value versus O_3 alone
Control	69.5 ± 13.9	—	0.012
Ozone	87.3 ± 4.6	0.012	—
Acid aerosol, 5 $\mu g/m^3$	64.7 ± 13.0	0.179	0.001
Ozone + 5 $\mu g/m^3$ aerosol	90.2 ± 15.0	0.026	0.263
Acid aerosol, 20 $\mu g/m^3$	70.1 ± 11.3	0.471	0.006
Ozone + 20 $\mu g/m^3$ aerosol	116.9 ± 6.4	<0.0001	<0.0001

[a]Data are mean values ± 1 SD ($n = 6$ rats per group) for total lung protein content of rats for the various exposure regimens. Data were analyzed for significance by Student's *t*-test; a value of $p \leq 0.05$ was taken as significant.

would be of interest to see if this finding is also true with sequential exposure regimens, but such experiments have not as yet been performed.

To further examine the range of concentrations of ozone and acid aerosol exhibiting synergistic interaction, rats were exposed to 0.20 ppm of O_3 in conjunction with 40 $\mu g/m^3$ of sulfuric acid aerosol. A synergistic interaction between 0.20 ppm of O_3 and 40 $\mu g/m^3$ of sulfuric acid was demonstrated by assay of tissue protein content after 7 or 9 days of exposure and by assay of lung collagen synthesis rate (*48*). We also found interactions between 0.20 ppm of ozone and 20 $\mu g/m^3$ of sulfuric acid, with an apparent no-observable effect level by assay of total lung protein content for sulfuric acid aerosol (plus 0.20 ppm of ozone) at or near 5 $\mu g/m^3$ (Table 5). The no-observable effect level for ozone (plus 0.5 mg/m^3 of acid aerosol) is at or below 0.12 ppm.

The acidic aerosol most likely to occur in polluted urban air under conditions of photochemical smog generation is ammonium sulfate or bisulfate, although ambient concentrations of these aerosols are far lower than those used in most of these experiments. We have previously shown that aerosols of neutral salts (sodium chloride or sodium sulfate) do not interact synergistically with O_3 (*38*). Furthermore, we have suggested that the acidity of an aerosol is the important determinant of whether the aerosol will synergistically interact with O_3 and have proposed a mechanism that suggests that the primary determinant for the elicitation of synergy between O_3 and acid aerosols is the aerosol acidity [and not the mere presence of the aerosol (*35*)]. If this mechanism is correct, then weak acids, such as ammonium sulfate, should cause less of a synergistic response than should strong acids, such as sulfuric acid, in accord with our observations in these studies (compare no-observable effect levels of 1 mg/m^3 or above for ammonium sulfate aerosol with about 5 $\mu g/m^3$ for sulfuric acid aerosol).

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